(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization International Bureau



(43) International Publication Date 15 July 2004 (15.07.2004)

PCT

(10) International Publication Number WO 2004/058787 A3

- (51) International Patent Classification7: C07F 9/6512, C07D 239/91
- C07H 15/26,
- (21) International Application Number:

PCT/EP2003/051105

(22) International Filing Date:

26 December 2003 (26.12.2003)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

02102898.0

27 December 2002 (27.12.2002) EP

- (71) Applicant (for all designated States except US): TI-BOTEC BVBA [BE/BE]; Generaal De Wittelaan L11 B3, B-2800 Mechelen (BE).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): GOEMAN. Jan, Ludwig [BE/BE]; Kleine Peperstraat 15, B-9100 Sint-Niklaas (BE). VAN ACKER, Koenraad, Lodewijk, August [BE/BE]; Uilenberg 30, B-9140 Temse (BE). VAN DER EYCKEN, Johan, Theo, André [BE/BE]; Denderhoutembaan 261, B-9400 Ninove (BE). DIERYNCK, Inge [BE/BE]; Moonsstraat 38, B-2018 Antwerpen (BE).
- (74) Agent: DAELEMANS, Frank; Tibotec-Virco Comm. VA, Generaal De Wittelaan L11 B3, B-2800 Mechelen (BE).

- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

of inventorship (Rule 4.17(iv)) for US only

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report: 20 January 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: FLUOROGENIC ENZYME SUBSTRATES AND METHODS OF PREPARATION

(57) Abstract: This invention relates to hydrolase fluorogenic substrates with improved cell permeability, methods for the preparation thereof, and methods of measuring activities of hydrolases, particularly in cell-based assays. The substrates easily diffuse into the cells, where they are enzymatically processed to yield photostable fluorescent products, and are particularly fitted for visualising enzyme-derived activities in cell-based assays.

International Application No PEP 03/51105

A. CLASS IPC 7	ification of subject matter C07H15/26 C07F9/6512 C07D239	/91	
	to International Patent Classification (IPC) or to both national classific	ation and IPC	
	S SEARCHED to commentation searched (classification system followed by classification system followed by cla	ion symbols)	
IPC 7	CO7H CO7F CO7D	,	
Documenta	ation searched other than minimum documentation to the extent that s	such documents are included in the fields so	earched
Electronic o	data base consulted during the international search (name of data be	ase and, where practical, search terms used	0
EPO-In	nternal, WPI Data, PAJ, MEDLINE, CHE	M ABS Data	
C. DOCUM	ENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the rel	levant passages	Relevant to claim No.
Х	WO 93/04077 A (MOLECULAR PROBES : 4 March 1993 (1993-03-04) abstract; claims 1-65; figure 1	INC)	1-12
Х	CHEMICAL ABSTRACTS, vol. 70, no. Columbus, Ohio, US; abstract no.: 77907, B.M.BOLOTIN ET AL.: "2-Aryl-4H-3,1-benzoxazin-4-ones analogs. II. Some Reactions of O-Acylaminobenzamides." XP002257309 abstract & ZH. VSES. KHIM. OBSHCHEST., vol. 13, no. 4, 1968, pages 475-4 registry number 22294-66-2 *	and their	1-5
X Furt	ther documents are listed in the continuation of box C.	Patent family members are listed in	n annex.
'A' docume consider the consider the consider the consider the constant of the	ategories of cited documents: ent defining the general state of the art which is not dered to be of particular relevance document but published on or after the international date ent which may throw doubts on priority claim(s) or is cited to establish the publication date of another nor other special reason (as specified) ent referring to an oral disclosure, use, exhibition or means ent published prior to the international filting date but han the priority date claimed actual completion of the international search	 *T' later document published after the Inte or priority date and not in conflict with cited to understand the principle or the invention *X' document of particular relevance; the cannot be considered novel or cannot involve an inventive step when the dor *Y' document of particular relevance; the cannot be considered to throubre an involve and the art. *&' document member of the same patent to the art. 	eory underlying the claimed invention to considered to current is taken alone laimed invention ventive step when the pre other such docurus to a person skilled tamily
1	5 November 2004	23/11/2004	
Name and r	malling address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo ni,	Authorized officer Scott. J	

International Application No
PEP 03/51105

		PO-7EP 03/51105
C.(Continu	ation) DOCUMENTS CONSIDERED TO BE RELEVANT	
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 2002, no. 05, 3 May 2002 (2002-05-03) & JP 2002 030085 A (ASAHI GLASS CO LTD;MITSUBISHI PHARMA CORP), 29 January 2002 (2002-01-29) abstract	1
Υ	Z.DIWU ET AL.: "Spectral Properties and Biological Applications of ELF Enzyme Substrates that yield Fluorescent Precipitates at the Enzymatic Activity Sites." PROCEEDINGS OF SPIE - THE INTERNATIONAL SOCIETY FOR OPTICAL ENGINEERING 3602 ADVANCES IN FLUORESCENCE SENSING TECHNOLOGY IV, 1999, pages 265-274, XP008023235 * the whole document, but especially the abstract; table 2, compound 19 *	1-12
Υ	WO 02/28841 A (MOLECULAR PROBES INC) 11 April 2002 (2002-04-11) the whole document	1-12
Υ	ZHENJUN D ET AL: "Fluorescent Molecular Probes I. The Synthesis and Biological Properties of an ELF@? beta-glucuronidase Substrate That Yields Fluorescent Precipitates at the Enzymatic Activity Sites" TETRAHEDRON, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 53, no. 21, 26 May 1997 (1997-05-26), pages 7159-7164, XP004105695 ISSN: 0040-4020 the whole document	1-12
	J.J.NALEWAY ET AL.: "Synthesis and Use of New Fluorogenic Precipitating Substrates." TETRAHEDRON LETTERS., vol. 35, no. 46, 14 November 1994 (1994-11-14), pages 8569-8572, XP002230449 ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM., NL ISSN: 0040-4039 the whole document	1-12

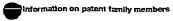
International Application No
POPEP 03/51105

	PO-FEP 03/51105
	10-1
Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
M.ZHOU ET AL.: "A Fluorogenic Substrate for beta-Glucuronidase: Applications in Fluorometric, Polyacrylamide Gel and Histochemical Assays." JOURNAL OF BIOCHEMICAL AND BIOPHYSICAL METHODS, vol. 33, 1996, pages 197-205, XP002230450 the whole document	1–12
US 5 443 986 A (HAUGHLAND RICHARD P ET AL) 22 August 1995 (1995-08-22) cited in the application abstract	1-12
EP 0 722 937 A (BAYER AG) 24 July 1996 (1996-07-24) example 6	1
US 4 159 330 A (CORNO MARIA L ET AL) 26 June 1979 (1979-06-26) * example 6, 6-carboxy-2-(2'-ethoxy- 5'-butyl-phenyl)-3,4-dihydro-4-oxo- quinazoline *	1
NL 6 409 642 A (AMERICAN CYANAMID COMPANY) 21 February 1966 (1966-02-21) * example 14: 3-butyl-2-(2-hydroxy phenyl)-4(3)-quinazoline *	
	for beta-Glucuronidase: Applications in Fluorometric, Polyacrylamide Gel and Histochemical Assays." JOURNAL OF BIOCHEMICAL AND BIOPHYSICAL METHODS, vol. 33, 1996, pages 197-205, XP002230450 the whole document US 5 443 986 A (HAUGHLAND RICHARD P ET AL) 22 August 1995 (1995-08-22) cited in the application abstract EP 0 722 937 A (BAYER AG) 24 July 1996 (1996-07-24) example 6 US 4 159 330 A (CORNO MARIA L ET AL) 26 June 1979 (1979-06-26) * example 6, 6-carboxy-2-(2'-ethoxy-5'-butyl-phenyl)-3,4-dihydro-4-oxo-quinazoline * NL 6 409 642 A (AMERICAN CYANAMID COMPANY) 21 February 1966 (1966-02-21) * example 14 : 3-butyl-2-(2-hydroxy



Integrational Application No PEP 03/51105

							03/51105
	document earch report	1	Publication date		Patent family member(s)		Publication date
WO 93	04077	Α	04-03-1993	US	5316906	5 A	31-05-1994
	0 10//	••	0. 00 1000	AT	144524		15-11-1996
				CA	2115461		03-12-1996
				DE	69214841		28-11-1996
				DE	69214841		28-05-1997
				EP	0641351		08-03-1995
				JP	8500961		06-03-1995
					3130316		
				JP	•		31-01-2001
				US	5443986		22-08-1995
				WO	9304077		04-03-1993
JP 20	02030085 	Α	29-01-2002	NONE			
WO 02	28841	Α	11-04-2002	AU	9650201		15-04-2002
				CA	2424300		11-04-2002
				EP	1322625		02-07-2003
				JP	2004510767		08-04-2004
				MO	0228841		11-04-2002
				US	2002137068	3 A1	26-09-2002
US 54	43986	Α	22-08-1995	US	5316906		31-05-1994
				ΑT	144524		15-11-1996
				CA	2115461		03-12-1996
				DE	69214841		28-11-1996
				DE	69214841	L T2	28-05-1997
				EP	0641351	L A1	08-03-1995
				JΡ	8500961		06-02-1996
				ĴΡ	3130316		31-01-2001
				WO	9304077		04-03-1993
EP 07	22937	Α	24-07-1996	DE	19501481	L A1	25-07-1996
				AU	704102		15-04-1999
				ΑU	4098096	5 A	25-07-1996
				BG	100293		31-07-1996
				BR	9600148		06-01-1998
				CA	2167345		20-07-1996
				CN	1134417		30-10-1996
				CZ	9600167		14-08-1996
				EE	9600019		15-08-1996
				ĔΡ	0722937		24-07-1996
				FI	960227		20-07-1996
				HR	960004		28-02-1998
				HU	960002		30-12-1996
					446776	·	
				IL	116//0		31-08-2000
				IN		–	07-10-2000
				JP	8253457		01-10-1996
				MA	23780		01-10-1996
				NO	960222		22-07-1996
				NZ	280831		28-10-1996
				PL	312352		22-07-1996
				RO	117451		29-03-2002
				RU	2158733		10-11-2000
				SG	34377	7 A1	06-12-1996
				SK	7796		07-08-1996
				TR	960709		21-08-1996
				US	5721238	3 A	24-02-1998



International Application No PeT/EP 03/51105

Patent document cited in search report	-	Publication date		Patent family member(s)	Publication date
US 4159330	Α .	26-06-1979	IT	1113561 B	20-01-1986
			IT	1118026 B	24-02-1986
			IT	1114419 B	27-01-1986
			AU	3134477 A	14-06-1979
			AU	512413 B2	09-10-1980
			BE	862201 A4	14-04-1978
			CA	1092118 A1	23-12-1980
			DE	2755716 A1	26-10-1978
			FR	2387967 A2	17-11-1978
			GB	1543874 A	11-04-1979
			GR	70041 A1	26-07-1982
			NL	7713862 A	23-10-1978
			NZ	185967 A	25-10-1979
			UY	300677 A1	31-08-1982
			ZA	7707410 A	25-10-1978
NL 6409642	Α	21-02-1966	NONE		